

COMPLETE LISTING OF CLAIMS IN APPLICATION

1. (withdrawn) A cytochrome P450 monooxygenase which is capable of at least one of the following reactions:

- a) oxidation of optionally substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds;
- b) oxidation of optionally substituted mono- or polynuclear aromatics;
- c) oxidation of straight-chain or branched alkanes and alkenes;
- d) oxidation of optionally substituted cycloalkanes and cycloalkenes;

where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88; except the single mutant Phe87Val.

2. (withdrawn) A monooxygenase as claimed in claim 1, which has at least one functional mutation in at least one of the sequence regions 73-82, 86-88 and 172-224.

3. (withdrawn) A monooxygenase as claimed in claim 1, which has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val, Leu188Gln; or
- b) Phe87Val, Leu188Gln, Ala74Gly;

and functional equivalents thereof which are capable of at least one of the above oxidation reactions.

4. (withdrawn) A nucleic acid sequence coding for a monooxygenase according to claim 1.

5. (withdrawn) An expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a coding sequence which comprises a nucleic acid sequence according to claim 4.
6. (withdrawn) A vector comprising at least one expression construct according to claim 5.
7. (withdrawn) A recombinant microorganism transformed by at least one vector as claimed in claim 6.
8. (withdrawn) A microorganism as claimed in claim 7, selected from bacteria of the genus *Escherichia*.
9. (currently amended) A process for the microbiological oxidation of N - or S - heterocyclic mono- or polynuclear aromatic compound which comprises
 - a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
 - a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium

, and wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO: 2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88.
10. (currently amended) A process as claimed in claim 9, wherein the exogenous or

intermediately formed substrate is selected from optionally substituted- N- or S-heterocyclic mono- or polynuclear aromatic compounds.

11. (canceled)

12. (currently amended) A process as claimed in claim 9 ~~11~~, where the mutant has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val;
- b) Phe87Val, Leu 188Gln; ~~or~~ and
- c) Phe87Val, Leu188Gln, Ala74Gly.

13. (withdrawn) A process for microbiological oxidation of optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises

- a1) culturing the recombinant cytochrome P450-producing microorganism as claimed in claim 7 in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
- a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88; and
- b) isolating the oxidation product formed or a secondary product thereof from the medium;

where the monooxygenase mutant Phe87Val is not excluded.

14. (withdrawn) A process as claimed in claim 13, wherein the exogenous or

intermediately formed substrate is selected from:

- a) optionally substituted mono- or polynuclear aromatics;
- b) straight-chain or branched alkanes and alkenes;
- c) optionally substituted cycloalkanes and cycloalkenes.

15. (canceled)

16. (withdrawn) A process as claimed in claim 13, where the cytochrome P450 monooxygenase has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val;
- b) Phe87Val, Leu188Gln; or
- c) Phe87Val, Leu188Gln, Ala74Gly.

17. (currently amended) A process as claimed in claim 9, wherein, as exogenous substrate, at least one compound selected from the groups a) to d) of compounds defined ~~above~~ claim 1 is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.

18. (original) A process as claimed in claim 17, wherein, as exogenous substrate, a compound selected from indole, n-hexane, n-octane, n-decane, n-dodecane, cumene, 1-methylindole, α -, β -, or γ -ionone, acridine, naphthalene, 6-methyl- or 8-methylquinoline, quinoline and quinaldine is employed.

19. (withdrawn) A process for the microbiological production of indigo and/or indixubin,

which comprises

- a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogeneous or intermediately formed indole; or
 - a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium.
20. (withdrawn) A process as claimed in claim 19, wherein the indigo and/or indirubin obtained, which was produced by oxidation of intermediately formed indole, is isolated from the medium.
21. (withdrawn) A process as claimed in claim 20, wherein the indole oxidation is carried out by culturing the microorganisms in the presence of oxygen at a culturing temperature of approximately 20 to 40°C and a pH of approximately 6 to 9.
22. (withdrawn) A process as claimed in claim 20, where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88, including the substitution Phe87Val.
23. (withdrawn) A process as claimed in claim 22, where the monooxygenase has at least one of the following mono- or polyamino acid substitutions:
- a) Phe87Val;

HAUER et al., Serial No. 10/031,146

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

24. (withdrawn) A bioreactor comprising the cytochrome P450 monooxygenase as claimed in claim 1 or a recombinant microorganism transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrome P450 monooxygenase of claim 1 in immobilized form.

25. (canceled)

26. (canceled)